FragSeq: transcriptome-wide RNA structure probing using high-throughput sequencing.

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Public Summary:
Understanding the way an RNA molecule interacts with itself through base-pairing interactions (termed RNA secondary structure) gives important information for how these molecules interact with other components in the cell including other RNA molecules, DNA and proteins. Such interactions have important regulatory consequences for gene expression and ultimately on cell fate and metabolism. Classical approaches to determine structures of non-coding RNA (ncRNA) probed ony one RNA at a time with enzymes and chemical, using gel electrophoresis to identify reactive positions. To accelerate RNA secondary structure inference, we developed fragmentation sequencing (FragSeq), a high-throughput RNA structure probing method that uses high-throughput RNA sequencing of fragments generated by digestion with nuclease P1, which specifically cleaves single-stranded nucleic acids. In experiments probing the entire mouse nuclear transcriptome, we accurately and simultaneously mapped single-stranded RNA regions in multiple ncRNAs with known structure. We probed in two cell types to verify reproducibility. We also identified and experimentally validated structured regions in ncRNAs with, to our knowledge, no previously reported probing data. FragSeq, combined with methods developed in previous RNA-seq studies, enables researchers to take high-throughput transcriptome analysis beyond one-dimensional sequence to reveal structural features of RNAs and provide clues to their underlying biology.

Scientific Abstract:
Classical approaches to determine structures of noncoding RNA (ncRNA) probed only one RNA at a time with enzymes and chemicals, using gel electrophoresis to identify reactive positions. To accelerate RNA structure inference, we developed fragmentation sequencing (FragSeq), a high-throughput RNA structure probing method that uses high-throughput RNA sequencing of fragments generated by digestion with nuclease P1, which specifically cleaves single-stranded nucleic acids. In experiments probing the entire mouse nuclear transcriptome, we accurately and simultaneously mapped single-stranded RNA regions in multiple ncRNAs with known structure. We probed in two cell types to verify reproducibility. We also identified and experimentally validated structured regions in ncRNAs with, to our knowledge, no previously reported probing data.